Oscillation of gap junction electrical coupling in the mouse pancreatic islets of Langerhans

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1. Pancreatic β-cells oscillate synchronously when grouped in islets. Coupling seems essential to maintain this oscillatory behaviour, as isolated cells are unable to oscillate. This allows the islet to be used as a model system for studying the role of coupling in the generation of oscillatory patterns.

2. Pairs of β-cells were intracellularly recorded in islets. β-Cells oscillated synchronously. Propagated voltage deflections were observed as a function of glucose concentration and of the distance between the recording electrodes. Space constants were smaller in the silent than in the active phases, suggesting a higher intercellular connection in the active phases.

3. Coupling coefficients and estimated coupling conductances were larger in the active than in the silent phases.

4. Coupling coefficients and coupling conductances changed dynamically and in phase with the membrane potential oscillations, pointing to an active modulation of the gap junctions.

5. We hypothesize a role for coupling in the generation of the oscillatory events, providing different levels of permeability dependent on the state of conductance during the oscillatory phases.

Oscillatory patterns are a common feature of excitable systems. In some cells the ionic conductances responsible for their excitability also endow them with autorhythmic electrical oscillatory properties (Llinas, 1988). In other systems it has been proposed that the oscillations arise from the association of the neurones' complement of ionic conductances and interneuronal electrotonic coupling (Yarom, 1989; Bleasel & Pettigrew, 1992). The understanding of the contribution of coupling conductances to oscillatory patterns has been hampered by the difficulties found in separating them from total membrane conductances. Nevertheless, it has been proposed that electrical coupling might provide, at least, a flexible mechanism for modifying the behaviour of an oscillatory neural network (Kepler, Marder & Abbott, 1990).

A potential case of coupling conductances participating in the emergence of an oscillatory pattern is seen in the pancreatic β-cell. It has been demonstrated, both *in vitro* and *in vivo*, that the electrical activity of β-cells is oscillatory in the physiological glucose concentration range (Sanchez-Andres, Gomis & Valdeolmillos, 1995; Valdeolmillos, Gomis & Sanchez-Andres, 1996). The demonstration of gap junctions between cells (Orci, Unger & Renold, 1973) prompted studies of their electrical coupling. Meissner (1976) showed that many of the β-cells from an islet are electrically coupled. The junction resistance was found to be modulated by glucose (Eddlestone, Gonçalves, Bangham & Rojas, 1984) and other natural and pharmacological secretagogues (Meda, Atwater, Gonçalves, Bangham, Orci & Rojas, 1984). Remarkably, the β-cells lose their oscillatory capability when isolated and recover it if they become clustered (Perez-Armendariz, Roy, Spray & Bennett, 1991; Smolen, Rinzel & Sherman, 1993). This behaviour leads us to hypothesize that the oscillatory pattern is an emergent property of the grouped β-cells, in that an isolated β-cell, with a complete repertoire of membrane conductances, requires appropriate feedback from neighbouring cells to exhibit oscillations. Considering the above, it has been proposed that bursting can be an emergent property of certain cell populations: cells which are not intrinsic bursters can burst when coupled (Sherman, 1994).

The presence of oscillatory activity, together with the geometrically simple sphere shape of the β-cells, makes the islet of Langerhans a good model system for studying the role of cell coupling in the generation of oscillatory patterns, and the synchronization mechanisms of heterogeneous cell populations.

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It has been reported that gap junctions are capable of being modulated by several extra- and intracellular agents (Spray, Harris & Bennett, 1981; Schmitz & Wolburg, 1991; Hampson, Vaney & Weiler, 1992; Barbosa, Salgado, Santos & Rosario, 1993; Bleasel & Pettigrew, 1994). It remains to be demonstrated whether the gap junction-mediated coupling conductance is constant or variable during the oscillations. This last possibility has been suggested, in part, on both theoretical and experimental bases (Kepler *et al.* 1990; Mears, Sheppard, Atwater & Rojas, 1995). If these resulted in being correct, it would be possible to consider the role of junctional coupling in the generation of oscillatory events, providing different levels of permeability to ions and intracellular messengers depending on the state of conductance along the oscillations.

The aim of this work was to determine whether the coupling conductance is fixed along a complete oscillatory cycle, or if it changes dynamically.

METHODS

The intracellular electrical activity of β-cells was recorded from microdissected islets of Langerhans obtained from albino mice, killed by cervical dislocation, as previously described (Sanchez-Andres, Ripoll & Soria, 1988). The experiments were carried out according to institutional animal care guidelines. The modified Krebs solution had the following composition (mM): 120 NaCl, 25 $NaHCO_3$, 5 KCl, 2·6 $CaCl_2$ and 1 $MgCl_2$, and was equilibrated with a gas mixture containing 95% O_2–5% CO_2 at 37 °C (pH 7·4).

An Axoclamp 2A microelectrode amplifier (Axon Instruments, Foster City, CA, USA) was used. Borosilicate microelectrodes (o.d., 2·0 mm; i.d., 1·0 mm; Sutter Instruments, Novato, CA, USA) were pulled with a Narishige PE2 puller (Narishige, Japan). The electrodes were filled with 3 M potassium citrate and 100 mM KCl. Data were acquired using Axotape software (v2.02, Axon Instruments) through an acquisition card (DMA-TL1, Axon Instruments), and stored on both computer hard disk and DAT magnetic tape for further analysis using Origin software (v3.73, Microcalc. Software, Northampton, MA, USA).

Pairs of cells ($n = 12$) were recorded as indicated in Fig. 1*A*. In order to keep the range of current injection linearity as broad as possible, only electrodes with an input resistance of less than 100 MΩ were used. After impalement, the bridge was balanced and a series of pulses was injected to check the range of linearity in the response of the electrodes (Fig. 1*B*). Electrodes with a narrow range of linearity were discarded before starting the current injection protocol. During the experiments, current values within the range of linearity of the electrodes were usually applied. Only pulses inside this range were quantified.

In experiments addressed to check coupling, cells were impaled in close vicinity to each other (distance between electrode tips, < 50 μm) to permit the quantification of induced voltage deflections. A mean of three cells interposed between the recorded cell pair could be assumed from a typical cell diameter of 10 μm. For convenience we will refer to the injected cell as cell 1, and the cell in which the corresponding propagated voltage deflection was measured as cell 2. If the electrodes were separated by distances greater than 50 μm, the deflections recorded in cell 2 were either not apparent, or were so small as to preclude suitable quantitative analysis, as has been previously described (Meda *et al.* 1984; Eddlestone *et al.* 1984).

Hyperpolarizing propagated deflections were fitted by a single-exponential curve. The time constant (τ) was measured in every experiment and ranged from 120 to 200 ms. Pulse duration was determined as a function of τ in every pair of cells. Pulses lasted for at least 4 times the duration of τ (600–900 ms) and measurements were made 30 ms before the end of the pulse. This procedure guaranteed that the steady-state values of the propagated voltage deflections had an error value of less than 5% when measured. The calculations in this paper were made from a mean of at least ten measurements obtained either in the silent or in the active phase. Hyperpolarizing current pulses were used throughout the study. Depolarizing pulses were not used so as to avoid the triggering of calcium action potentials, which would result in the inaccurate measurement of the propagated voltage deflections. Deflections in the active phase were eventually distorted by residual spiking. In these cases measurements were discarded if the exponential course was masked in such a way as to make them inaccurate.

Coupling coefficients and coupling resistance measurements

A major constraint in quantifying a value for the coupling conductance between cells is the contamination from other

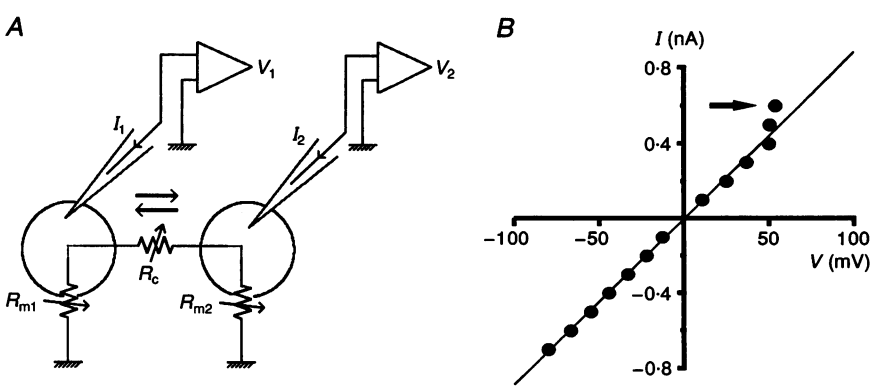

Figure 1. Experimental procedures

A, the basic circuitry of the experimental procedure. V_1 and V_2, membrane potentials recorded in cell 1 and cell 2, respectively; I_1 and I_2, current injected in cell 1 and cell 2, respectively; R_c, junction resistance; R_{m1} and R_{m2}, non-junction resistances of cell 1 and cell 2, respectively. *B*, the response of a typical electrode. Arrow indicates the level of injected current required to drive the response of the electrodes out of linearity.

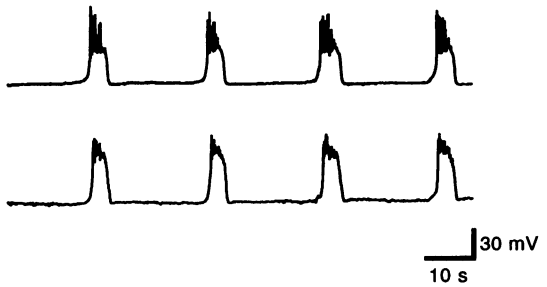

Figure 2. Simultaneous records from two cells in the same islet

Representative pair of two simultaneously recorded cells. Glucose concentration, 11·1 mM. Both cells oscillated synchronously.

membrane conductances. Bennett (1966) proposed a model for electrotonic junctions based on an equivalent circuit of two connected cells. Bennett (1966) defined the coupling coefficient ($k_{1,2}$) for a pair of cells as the ratio of the potential (V_2) produced in one cell (cell 2) by the potential (V_1) applied to the other (cell 1), and demonstrated the relationship of these potentials with the non-junctional cell resistances (R_{m1} and R_{m2}, for cell 1 and cell 2, respectively) and the coupling resistance (R_c).

We recorded V_1 and V_2 and the corresponding input resistances ($R_{1,1}$ and $R_{2,2}$) in pairs of simultaneously impaled cells in order to calculate their coupling coefficients and the values for R_{m1}, R_{m2} and R_c (Bennett, 1966).

Statistical analysis

A Pearson correlation analysis was carried out using Instat2 software (v2.04, GraphPAD Software Inc., San Diego, CA, USA).

RESULTS

At glucose concentrations lower than 7 mM the β-cell membrane potential remained steadily hyperpolarized. The electrical pattern of the recorded β-cells consisted of periodic membrane potential oscillations when the bath glucose concentration ranged from 7 to 20 mM. The strict periodicity of the membrane oscillations recorded in this glucose range allowed us to consider two phases of electrical activity: the active or depolarized phase, and the silent or hyperpolarized phase (Fig. 2) as previously described (Dean & Matthews, 1968). Pairs of cells simultaneously impaled in the same islet showed synchronous behaviour, in that the active and silent phases strictly coincided, agreeing closely with previous observations (Meissner, 1976; Valdeolmillos *et al.* 1996) (Fig. 2). Typically, the depolarization of the

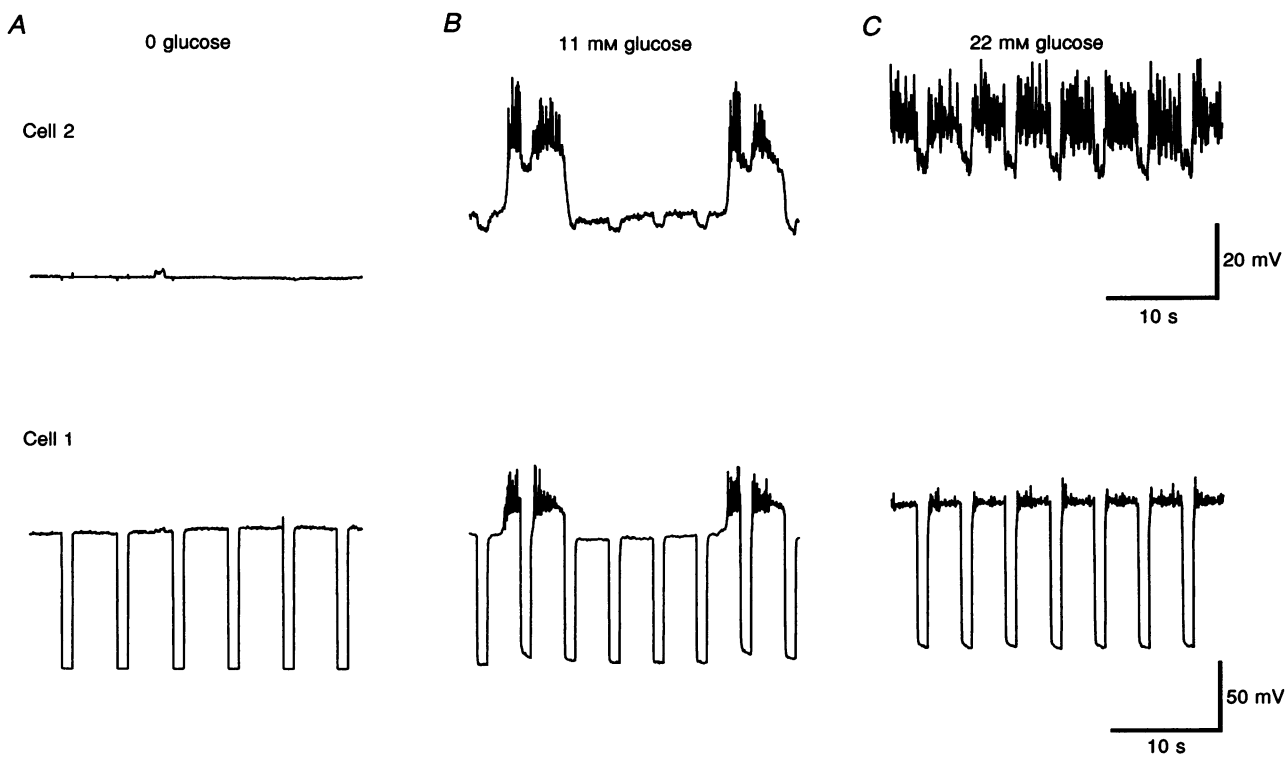

Figure 3. Glucose dependence of intercellular coupling

A, in the absence of glucose, current injected into cell 1 is unable to produce propagated voltage deflections in cell 2. *B*, identical current pulses injected in the presence of glucose (11 mM) determined the presence of measurable voltage deflections, which became greater as the glucose concentration was increased (*C*, 22 mM).

active phase drove the membrane potential to a threshold level, which triggered voltage-dependent calcium spikes. Increasing the glucose concentration resulted in a lengthening of the active phases at the expense of the silent ones. At glucose concentrations greater than 20 mM the oscillatory pattern disappeared and the cells fired continuously at a relatively depolarized membrane potential (Fig. 3).

Coupling between cells is not constant and changes as a function of glucose concentration, which drives the metabolic state of the cells and their membrane potential. In the absence of glucose, current injected into cell 1 is unable to induce propagated deflections in cell 2 (Fig. 3*A*). Higher glucose concentrations (11 and 22 mM; Fig. 3*B* and *C*) play a permissive role in allowing propagated voltage deflections to be observed in cell 2. Similar observations have also confirmed that glucose concentration increases coupling in the pancreatic β-cells (Meda *et al.* 1984).

Cable properties of the islet cells

Current propagation between islet cells not only depends on glucose concentration but also on cell topography. If the electrodes impaling two cells are relatively close to each other (i.e. < 50 μm apart), it is possible to observe propagated voltage deflections in cell 2. If the cell 2 electrode is located further away, propagated deflections are not observed. The absence of a quantifiable response in cells separated by longer distances was attributed to the electrotonic decay of the injected pulses, rather than to the absence of coupling, since in 90% of the cases the cells showed synchronic oscillations (Fig. 4*A*, left), independent of the relative topography of the simultaneous impalements. This result is consistent with the passive decay associated with the resistive–capacitive circuit provided by intercellular connections. This hypothesis has already been tested showing that there is electrical coupling between islet cells and a space constant of the coupling ratio of the order of a few β-cell diameters. We have carried out a multi-electrode analysis in order to assess the validity of our data.

Plots of the voltage deflections induced in cell 2 (V_2) by current injected into cell 1 (I) as a function of the distance between cells are fitted well by single-exponential curves (Fig. 4*B*). Curves and their corresponding time constants in the silent and active phases are different. The time constants give the values for the propagation decay (space constants): 41 μm in the active phase and 33·7 μm in the silent phase. It is worth noting that the curves start at a constant displacement of 20 μm on the x-axis, which corresponds to the minimal intercellular distance estimated from the mean cell diameter (2×10 μm). Taking this correction into consideration, the actual space constants will be 21 and 13·7 μm, respectively. The difference in the space constants strongly suggests a change in the junction properties associated with the phase of the oscillation. To gain further insight we have analysed the coupling coefficients along the oscillatory phases.

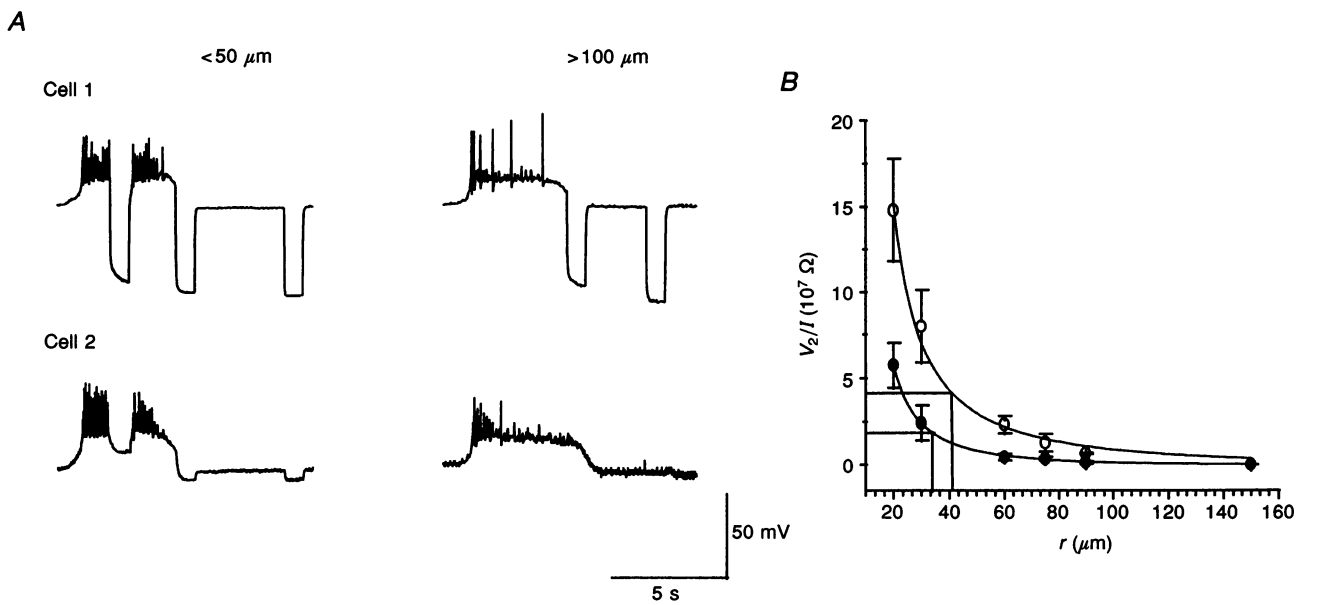

Figure 4. Cable properties of the system

A, pulse propagation is a function of the distance between impaled cells. If the electrodes are placed less than 50 μm apart, propagated deflections can be observed in cell 2 as a result of current injected into cell 1. At distances greater than 50 μm between electrodes propagated deflections are hardly observed, and at 100 μm no longer detected. *B*, plot of deflections induced in cell 2 (V_2) by current injected into cell 1 (I) *vs.* the distance (r) between both cells. The decline of the propagated signals, as a function of distance, are fitted well by single-exponential curves. The functions and corresponding space constants are different for the active (○) and silent phases (●).

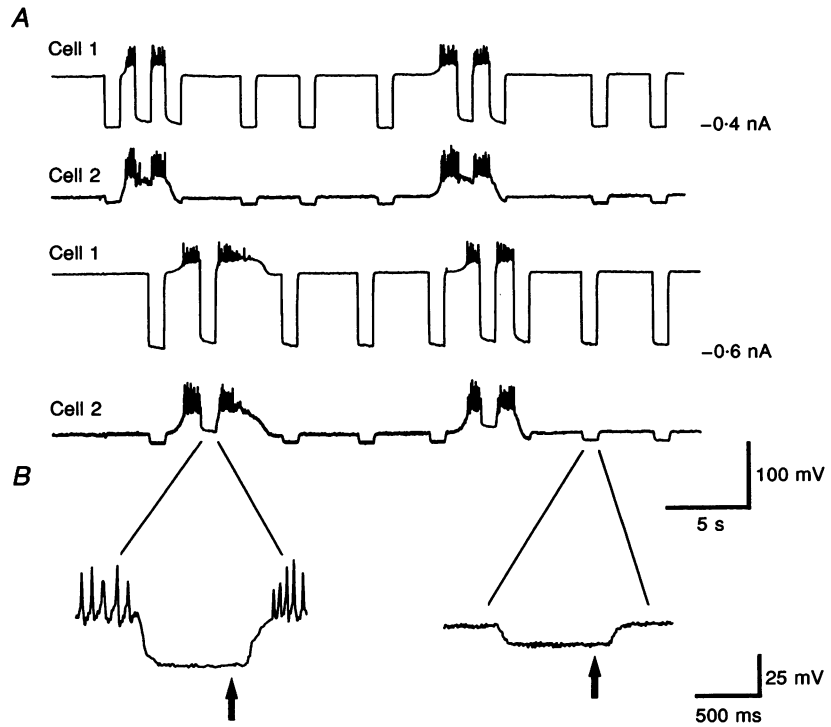

Figure 5. Intercellular propagation of the pulses depends on the phase of oscillation
A, current pulses (−0·4 and −0·6 nA) injected into cell 1 induced propagated voltage deflections in cell 2. These deflections had greater amplitude in the active phases than in the silent phases. In both phases the amplitudes were a function of the current injected. *B*, two sample deflections recorded in cell 2 during the active and silent phase magnified. Arrows indicate the points where measurements were made.

Coupling coefficients along the oscillatory phases

In Fig. 5, we show the simultaneous records from two cells. In both the silent and active phases the amplitude of voltage deflections in cell 2 increased linearly as the injected current at cell 1 was increased (Fig. 6*A*, continuous lines). If the differences in the voltage deflections recorded during the silent and active phases were a consequence of changes in input resistance, it would be possible to estimate ohmically the expected deflections in the active phases by multiplying the mean value of the deflections in the silent phases by a coefficient corresponding to the increase in input resistance in the active phases (Fig. 6*A*, interrupted line). The discrepancy

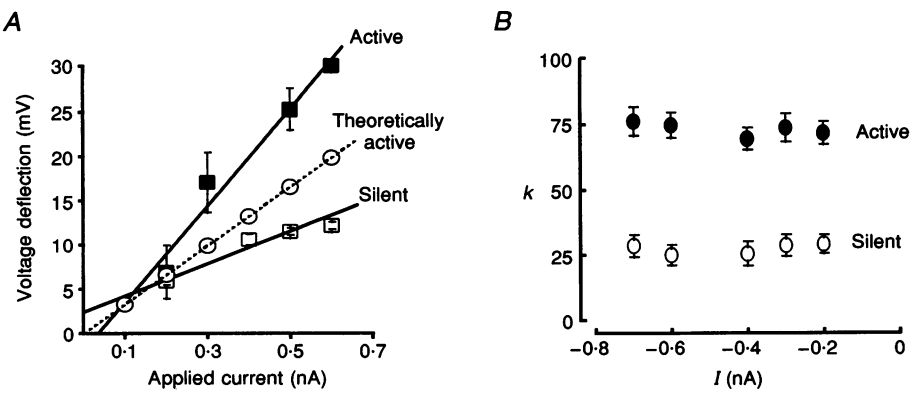

Figure 6. Dependence of cell 2 voltage deflections and coupling coefficients on cell 1-injected current
A, plot of voltage deflections induced in cell 2 *vs.* current pulses applied to cell 1. □, experimental values obtained during the silent phases. ○ and interrupted line, estimated values for the active phases. ■, experimental values obtained during the active phases. Silent phase input resistance, 210 MΩ; active phase input resistance, 298 MΩ. *B*, the coupling coefficients (*k*) for the silent and active phases.

Table 1. Ratios of the deflections in active and silent phases ($\Delta V_A/\Delta V_S$)

Current injected into cell 1	Current injected directly into cell 2
6·31	1·19
3·73	1·27
5·50	1·18
6·90	1·13
2·42	1·22
2·47	1·15

Current was either injected into cell 1 of a pair and recorded in cell 2 (left column), or directly injected into cell 2 (right column).

between estimated and experimental values suggests a contribution by the intercellular coupling conductance. This is further supported by the difference observed in the ratio of deflections in the active and silent phases ($\Delta V_A/\Delta V_S$) obtained when current was directly injected into cell 2 or coming from cell 1 (Table 1). Directly injected current can be assumed to be constant, the ratio reflecting changes in cell input resistance. Current coming from cell 1 goes through the coupling connections, and consequently, the ratio reflects their state of conductance and cell input resistance. If the conductance between cells during the phases of the oscillations did not vary, we would expect similar ratios from both sources of current. Table 1 shows that the ratios obtained after direct current injection into cell 1 are, on average, 3·85 times greater than those obtained from direct injection into cell 2.

We evaluated the contribution of the coupling conductance by calculating the coupling coefficients ($k_{1,2}$) as described in Methods. Figure 6*B* shows that the coupling coefficients are greater in active than in silent phases, and in both states are independent of the current injected. The estimated values for coupling conductances (G_c) were 514 ± 137 pS (G_c ± S.D.) in the active phases and 149 ± 41 pS in the silent phases.

No significant changes in the coupling coefficients were observed during the non-oscillatory states of the cells (glucose concentration, < 7 or > 20 mM) (data not shown).

Phase dependence of coupling conductance oscillations in relation to membrane potential oscillations

The above data suggest a change in the conductance between cells as they oscillate. Pearson correlation analysis was used on data from six cells to check the correlation between changes in the coupling coefficients and oscillations in membrane potential. The Pearson coefficients obtained ranged from 0·85 to 0·99 (Table 2). Figure 7*A* shows that the points in the correlation plot are concentrated in two clusters that correspond to the voltage levels of the silent and active phases. This is illustrated further in Fig. 7*B* and *C*, where the coupling coefficients, obtained from several successive oscillations of a single cell with a high degree of periodicity, were superimposed. These results strongly suggest that changes in the intercellular connection are oscillatory and take place in phase with oscillations of the membrane potential.

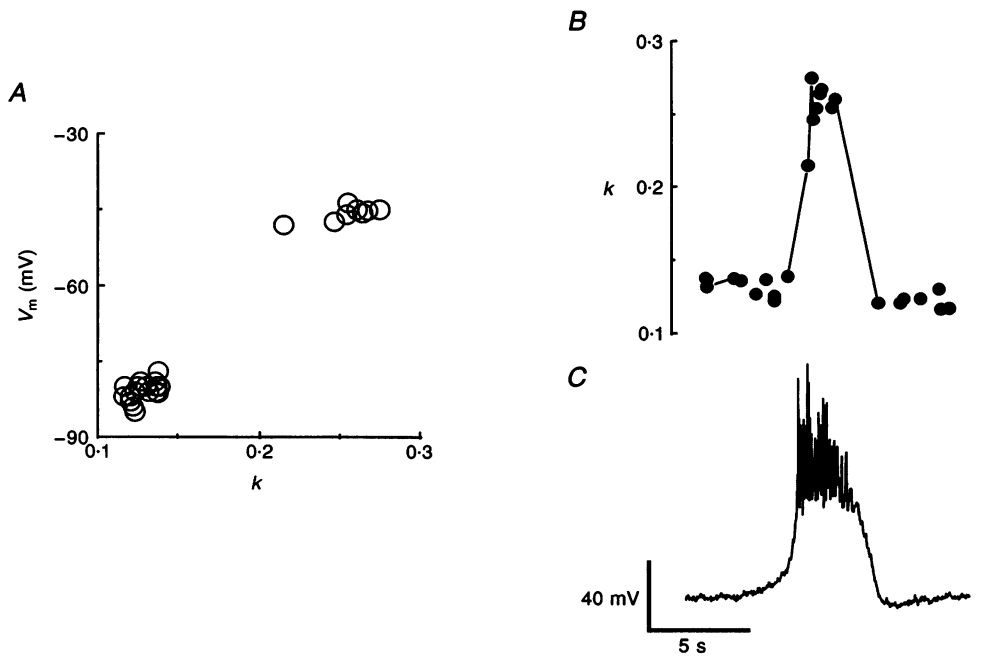

Figure 7. Phase dependence of the changes in intercellular connection

A, correlation plot of the membrane potential (V_m) *vs.* coupling coefficient (k) (Pearson correlation, $r = 0·98$; $P < 0·0001$). *B*, coupling coefficients measured from nine consecutive oscillations in cell 2 were superimposed. Time = 0 was arbitrarily assumed as the moment of initial depolarization towards the active phase. *C*, representative oscillation from the cell used in *B*.

Table 2. Pearson correlation analyses for changes in the coupling coefficients during membrane potential oscillations

r	95% confidence interval
0·98	0·95–0·99
0·85	0·67–0·94
0·87	0·74–0·95
0·95	0·87–0·98
0·97	0·94–0·99
0·95	0·88–0·98

r is the Pearson correlation.

DISCUSSION

A correlation between the onset of oscillations and a decrease in apparent membrane resistance, which might reflect the establishment of electrotonic coupling, has been reported in developing inferior olivary neurones. Furthermore, other studies of these neurones support the hypothesis that oscillations of the membrane potential depend not only on specific membrane conductances but also on electrotonic coupling between neurones (Bleasel & Pettigrew, 1992). These facts point to the participation of coupling conductances in the establishment of oscillatory activity. Nevertheless, it remained to be demonstrated whether the contribution of coupling conductance to the total input resistance was constant, or if it varied between the phases of the oscillations. This point seemed potentially relevant on two counts: firstly, in the islet of Langerhans it appears that coupling is the major factor in explaining the differences in behaviour of isolated cells when compared with clustered cells; secondly, characterization of the role of the coupling conductance in this relatively simple system could be useful in understanding the role of coupling conductance in geometrically more complex oscillatory systems. The data presented here show differences in the propagated voltage deflections measured in cells other than those injected. These differences are characterized as follows: (i) during both silent and active phases, the amplitude of propagated voltage deflections increases with the amount of current injected into cell 1; (ii) the amplitude of deflections is greater during the active phase than during the silent one; and (iii) the size of deflections during the active phase deviates from the estimated values calculated from the input resistance. These differences in size of recorded deflections in cell 2 cannot be solely accounted for by changes in the input resistance of the cells (Fig. 6), as can be seen from a comparison of the $\Delta V_A/\Delta V_S$ ratios from the active and silent phases. If the differences in propagated voltage deflections were a consequence of changes in input resistance, one would expect the same ratios for deflections measured during the silent and active phases in cell 2 independently of the source of current. On the contrary, however, our data show that the ratios are almost 4 times higher when current had to pass through cell junctions (Table 1).

Analysis of the cable properties of the system seemed to indicate a change in coupling conductance along the oscillation phases (Fig. 4). Different space constants were obtained in the silent and active phases, the active phases having the higher values. This finding implies the faster spatial decay of a propagated signal in the silent phases, and an increased degree of coupling in the active ones. Testing this possibility requires the analysis of coupling properties in both phases of oscillation. Bennett (1966) has described a broadly accepted procedure to quantify coupling in sphere-shaped cellular systems, which consists of calculating the coupling coefficients as already described in the Methods. We have calculated the coupling coefficients and shown that they are higher in the active than in the silent phases of the oscillations, therefore suggesting a higher level of intercellular connection during the active phases. In both cases the coupling coefficients were independent of the current amplitude injected into cell 1 (Fig. 6*B*) and therefore independent of the absolute values of the measured voltage deflections. The constancy of the coupling coefficients explains the linearity in voltage responses shown in Fig. 6*A*, and could have been expected as a consequence of the voltage-independent coupling previously described in this system (Perez-Armendariz *et al.* 1991). The coupling conductances estimated from the coupling coefficients (active phase, 514 ± 137 pS; silent phase, 149 ± 41 pS) (Table 1) were in the range already determined using the double whole-cell patch clamp technique (215 ± 110 pS) (Perez-Armendariz *et al.* 1991).

Furthermore, we have shown that the coupling coefficient changes in phase with membrane potential oscillations (Fig. 7). We propose that coupling is not constant but that it oscillates between active and silent phases. Interestingly, this coupling oscillation is not voltage dependent, (Eddlestone *et al.* 1984; Perez-Armendariz *et al.* 1991), indicating that these changes cannot be accounted for by the different voltages in the silent and active phases.

Such dynamic changes could be mediated by intra- or extracellular agents, given that the connecting molecules had an appropriate sensitivity. Local changes in the vicinity of the gap junctions cannot be excluded. Potential extracellular candidates are several secretagogues, which are able to modulate the junctional resistance (Meda *et al.* 1984), and other molecules known to oscillate with a burst pattern, like K^+, Ca^{2+} (Perez-Armendariz & Atwater, 1986), or insulin (Rosario, Atwater & Scott, 1986). It has been published that junctional resistance is modulated by glucose and other natural and pharmacological secretagogues (Meda *et al.* 1984). In fact, it has been reported that coupling depends on glucose concentration (Eddlestone *et al.* 1984), but our results cannot be explained on this basis, as the glucose concentration was kept constant throughout the experiment. Other agents are able to affect coupling, such as dopamine, which uncouples horizontal cells in the retina (Spray & Bennett, 1985; Marty,

1987; Kepler *et al.* 1990). The same effect has been observed in amacrine cells, mediated by the stimulation of cAMP production (Hampson *et al.* 1992). Potential intracellular candidates for mediating the coupling oscillation are pH and $[Ca^{2+}]$. pH is a well-known modulator of gap junctions (Spray *et al.* 1981; Bleasel & Pettigrew, 1994), and it has been reported that in β-cells, the oscillatory properties are closely dependent on the intracellular pH (Barbosa *et al.* 1993). Intracellular $[Ca^{2+}]$ has been shown to oscillate between 50 and 300 μM, in parallel with the oscillations of membrane potential (Santos, Rosario, Nadal, García-Sancho, Soria & Valdeolmillos, 1991).

The role of intrinsic cell conductances in the generation of oscillatory patterns has been well characterized (Llinas, 1988), but it has been predicted on a theoretical basis that coupling conductances also have an active contribution (Kepler *et al.* 1990). The relative weight of each components is a matter for discussion. In pancreatic β-cells it seems that ionic conductances constitute a necessary, but not sufficient condition, since isolated cells do not oscillate, but will do if they become clustered. This type of evidence leads us to propose that bursting is an emergent property of populations: cells which are not intrinsic bursters can burst when coupled (Sherman, 1994). Junction coupling can be substantial, providing different levels of permeability to ions and intracellular messengers, as a result of the different levels of conductance along the oscillations. Furthermore, the oscillation of coupling conductance in phase with the oscillations of membrane potential can contribute to the electrical synchronization of gap junction-connected cell networks, which agrees well with the proposal of electrical coupling as a mechanism for synchronizing electrical activity within the islet (Perez-Armendariz *et al.* 1991). Provided this hypothesis is correct, neuromodulatory substances that change the level of electrical coupling may have complex effects on the emergent frequency of an oscillatory network (Spray & Bennett, 1985; Marty, 1987; Kepler *et al.* 1990).

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